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# CDC42 and Rac1 Control Different Actin-dependent Processes in the *Drosophila* Wing Disc Epithelium

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*Abstract.* Cdc42 and Rac1 are members of the rho family of small guanosinetriphosphatases and are required for a diverse set of cytoskeleton–membrane interactions in different cell types. Here we show that these two proteins contribute differently to the organization of epithelial cells in the *Drosophila* wing imaginal disc. Drac1 is required to assemble actin at adherens junctions. Failure of adherens junction actin assembly in Drac1 dominant-negative mutants is associated with increased cell death. Dcdc42, on the other hand, is required for processes that involve polarized cell shape changes during both pupal and larval development. In the third larval instar, Dcdc42 is required for apico-basal epithelial elongation. Whereas normal wing disc epithelial cells increase in height more than twofold during the third instar, cells that express a dominant-negative version of Dcdc42 remain short and are abnormally shaped. Dcdc42 localizes to both apical and basal regions of the cell during these events, and mediates elongation, at least in part, by effecting a reorganization of the basal actin cytoskeleton. These observations suggest that a common cdc42-based mechanism may govern polarized cell shape changes in a wide variety of cell types.

A cell's ability to polarize the organization of its various protein and membrane components is critical to the performance of its specialized functions and maintenance of the differentiated state. Epithelial cells are a particularly well-studied example of such polarity. These cells form boundaries separating their basolateral and apical environments and regulate the passage of substances between them (Simons and Fuller, 1995; Rodriguez-Boulan and Nelson, 1989).

Epithelial cells organize themselves in response to positional cues from their environment. Interaction with basal extracellular matrix proteins and with other epithelial cells are each thought to contribute to the determination of an apical basal axis (Brower and Jaffe, 1989; Wang et al., 1990; Ojakian and Schwimmer, 1994; van Adelsberg et al., 1994). These interactions result in the formation of multiprotein junctional complexes that link extracellular positional cues to the actin cytoskeleton (Gumbiner and McCrea, 1993). These junctions and their connection to the cytoskeleton are mainstays of epithelial organization (Näthke et al., 1993). In vertebrate cells, alterations in adherens junction proteins are often associated with loss of epithelial morphology and transformation (Behrens et al., 1989, 1993; Kawanishi et al., 1995). Furthermore, characterization of the *Drosophila* disc overgrowth mutants, which cause hyperplasia and loss of epithelial morphology in disc epithelia, has emphasized the importance of intercellular junctions and the cytoskeleton to the maintenance of a polarized epithelium (Jursnich et al., 1990; Woods and Bryant, 1991; Strand et al., 1994*a*,*b*).

The process of polarization and its effect on cellular architecture has been well studied in the MDCK epithelial cell line (Bacallao et al., 1989; Buendia et al., 1990). The polarity of MDCK cells can be disrupted by trypsinization during passaging and reestablished as cell contacts reform (Balcarova-Stander et al., 1984). When MDCK cells make contact, actin relocates from the perinuclear region and forms both an apical circumferential band underlying the junctional region and basal stress fibers. Once intercellular junctions have been established, the cells begin to elongate apico-basally. Concomitantly, centrioles separate and migrate apically, and microtubules are reorganized, changing from a radial to a polarized longitudinal array with basally oriented plus ends. Centriole separation and microtubule reorganization probably depend on junctional microfilaments because treatment with either cytochalasin D or low calcium disrupts this process.

Since actin reorganization is implicated in the execution of many different aspects of polarization, we would like to understand its role in greater detail. To do this, it would be desirable to disrupt a specific subset of actin-dependent structures without affecting other aspects of the cytoskeleton. One way to perturb particular aspects of the actin cytoskeleton might be to disrupt the function of specific rho

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family proteins. The rho family of small GTPases is highly conserved between phyla and regulates a variety of actin-dependent processes in different cell types (Adams et al., 1990; Chant and Herskowitz, 1991; Ridley and Hall, 1992; Ridley et al., 1992; Luo et al., 1994; Miller and Johnson, 1994; Nishiyama et al., 1994; Nobes et al., 1995; Kozma et al., 1995). These processes include budding and shmooing in yeast, membrane ruffling, filopodium formation, stress fiber formation, neurite outgrowth, and myoblast fusion. Despite extensive study of these proteins in other cell types, their functions have not yet been addressed in epithelial cells. We decided to investigate the role of two of these proteins, Dcdc42 and Drac1, in epithelial organization.

The epithelium we have chosen as a model system is the *Drosophila* wing imaginal disc. Imaginal discs are epithelial tissues that generate the final shape of the adult and secrete the adult cuticle (Poodry, 1980). They derive from invaginations of the embryonic ectoderm, and while the rest of the animal becomes polyploid during the larval instars, these cells remain diploid and continue to divide. As they increase in size they form folded, relatively undifferentiated epithelial sacs that remain connected to the larval ectoderm by a thin stalk. In response to an increase in ecdysone titer that marks the beginning of pupal development, the discs unfold and assume the shape of the appendages to which they will give rise.

Since Dcdc42 and Drac1 have been shown to play important roles in many cell types, mutations in these genes are likely to have pleiotropic effects. We therefore decided to study Dcdc42 and Drac1 function by targeting the expression of dominant-negative alleles of these genes to a specific subset of disc epithelial cells. Dominant-negative and constitutively active mutations, which stabilize the GDP- and GTP-bound states, respectively, have been made by analogy with the ras mutations and are well characterized. The expression of a dominant-negative mutant protein is thought to render the endogenous protein inactive by sequestering its interaction partners in nonproductive complexes. Conversely, constitutively active proteins are functional but unregulatable (Barbacid, 1987). In these studies, we use the dominant-negative mutations S89 and N17 and the constitutively active mutant V12. These mutations have been used in many cell types to examine the function of cdc42 and rac1 (Ridley et al., 1992; Luo et al., 1994; Nobes et al, 1995; Harden et al., 1995; Kozma et al., 1995).

## Materials and Methods

### Flies

Flies were raised on a cornmeal, yeast, and molasses medium at 22°C. To generate discs in which mutant Dcdc42 and Drac1 alleles were expressed at the compartment boundary, we crossed females harboring a construct that expressed the Dcdc42 or Drac allele under the control of the *gal4*UAS (Luo et al., 1994) with males that expressed gal4 under the control of the patched promoter (Hinz et al., 1994). Flies laid eggs for 2 d at 18°C, and development proceeded at 18°C for another 24 h. The larvae were then shifted to 22°C and fed for ~4 d. This procedure was followed to maximize survival through embryonic and early larval stages. The cloning of Dcdc42 and Drac1, which map to the X chromosome and 61F, respectively, has been previously reported (Luo et al., 1994).

### Antibodies

Antibodies against murine cdc42 were prepared by immunizing chickens with a cdc42–glutathione S transferase (GST) fusion protein. Egg extracts were first passed over a GST column and the flowthrough applied to a column containing cdc42 fused to GST. The resulting affinity-purified antibody was used at a dilution of 1:20. Antibodies against *Drosophila* rac1 were made by immunizing rabbits with a peptide corresponding to amino acids 124–150 (CNTIEKLRDKKLVPITYPQGLAMAKEIG) of the Drac1 protein (Luo et al., 1994). The peptide was synthesized by Dr. C. Turck (Howard Hughes Medical Institute, University of California at San Francisco). It was coupled via its $NH_2$-terminal cysteine to keyhole limpet hemocyanin and injected into rabbits using standard procedures for antiserum production by CALTAG Corporation (South San Francisco, CA). The antibody was affinity purified with a peptide column and used at a dilution of 1:200. β-integrin was detected with an antibody produced in rabbits against chicken β-integrin (Marcantonio and Hynes, 1988). This antibody detects the *Drosophila* β-integrin protein encoded by the *myospheroid* gene and was used at a dilution of 1:200. The rat monoclonal antibody against *Drosophila* E-cadherin (Oda et al., 1994) was used at a dilution of 1:10. The affinity-purified anti–armadillo antibody (Riggleman et al., 1990) was used at a dilution of 1:200. The rabbit antiyellow antibody (Kornezos and Chia, 1992) was used at a dilution of 1:1,000. The affinity-purified rabbit anti–armadillo antibody was used at a dilution of 1:200.

### Immunofluorescent Staining and Light Microscopy

Wing discs were dissected in Shield and Sang's M3 medium. They were kept at room temperature until sufficient numbers of discs had been collected (a maximum of 30 min).

The fixation procedure varied depending on the antigen. For staining with phalloidin alone, or phalloidin and anticadherin antibody, discs were fixed for 10 min in a buffer containing 8% formaldehyde, 100 mM K cacodylate, pH 7.2, 100 mM sucrose, 40 mM K acetate, 10 mM Na acetate, and 10 mM EGTA. Discs were then rinsed three times in PBT[1] (PBS and 0.1% Triton X-100) and permeabilized for 1–2 h in PBS and 1% Triton X-100. After permeabilization, the discs were washed three times for 5 min in PBT, then incubated with 2 U/ml rhodamine phalloidin (Molecular Probes, Inc., Eugene, OR) either for 10 min (when staining with phalloidin alone) or throughout the rest of the antibody-staining procedure (when staining with both phalloidin and anti-cadherin antibody). This procedure gave the best preservation of the basal actin cytoskeleton.

For double staining with phalloidin and anti–armadillo or phalloidin and anti-Drac1 antibody, discs were fixed in a buffer containing 0.1 M Pipes, pH 6.5, 1 mM EGTA, 2 mM $MgSO_4$ (PEM) and 1% NP-40 and 1% formaldehyde for 30 min, then washed three times for 5 min in PBN (PBS and 0.1% NP-40).

For staining with anti-yellow or anti-integrin antibodies, discs were fixed for 10 min in PEM and 4% paraformaldehyde, then washed three times for 5 min in PBT.

For staining with anti-cdc42 antibody, discs were fixed for 5 min in 0.1 M Pipes, 1 mM EGTA, 2 mM $MgSO_4$, and 4% paraformaldehyde (diluted from a freshly prepared 20% stock), rinsed once with PBS, rinsed once with ice-cold methanol, and then refixed for 5 min in ice-cold methanol. Discs were then washed three times for 5 min in PBT.

After blocking in 5% normal goat serum for 30 min, binding of primary antibodies was routinely performed in either PBT or PBN (as described above) overnight at 4°C, excepting anticadherin, which was bound for 2 h at room temperature. After binding, the discs were rinsed three times in PBT or PBN, washed three times for 15 min, and blocked for 30 min in normal goat serum. Fluorescently labeled or biotinylated secondary antibodies were preabsorbed with fixed embryos at a 1:10 dilution overnight at 4°C, and then diluted 20–50-fold and incubated with discs for 1 h. After binding, discs were rinsed three times in PBT or PBN and washed three times for 15 min. When biotinylated secondary antibodies were used, this was followed by a 5-min incubation with fluorescently labeled streptavidin and another series of washes. Discs were then rinsed in PBS and placed in mounting medium (87% glycerol, 10 mM Tris, pH 8.5, 4% propyl gallate, final pH adjusted to 8.5). In cases where we wished to focus on a particular side of the disc (apical or basal), we turned them so that that side faced the coverslip to increase the resolution in that region. Coverslips were perched on thin bridges cut from cellophane and sealed with nail polish.

Discs were observed in a confocal microscope built at EMBL (Stelzer et al., 1989).

1. *Abbreviations used in this paper*: AP, anterior-posterior; GST, glutathione S transferase; PBN, PBS plus 0.1% NP-40; PBT, PBS plus 0.1% Triton X-100.

### *Detection of β-Galactosidase by X-gal Staining*

Discs were fixed as described below, washed in PBS, then incubated at room temperature in a solution containing 30 mM $Na_2HP0_4$, 12 mM $NaH_2PO_4$, 150 mM NaCl, 1 mM $MgCl_2$, 3 mM $K_3(Fe(CN)_6)$, 3 mM $K_4(Fe(CN)_6)$, and 0.2% X-gal.

### *Histological Sections and EM*

Discs were processed for EM according to a protocol similar in most respects to that described in Jursnich et al. (1990). Discs were dissected as described above and fixed in a buffer containing 0.1 M Na cacodylate, pH 7.4, 100 mM sucrose, and 2.5% glutaraldehyde for 2 h at room temperature. Discs were then washed twice for 5 min in 0.1 M Na cacodylate and 100 mM sucrose and fixed overnight at 4°C with 1% osmium tetroxide in 0.1 M Na cacodylate and 100 mM sucrose. If the samples were to be stained with X-gal, we first fixed for only 2 min under these conditions, stained with X-gal as described above, and then continued fixation for 2 h. We based this protocol on that described in Stollewerk and Campos Ortega (1995). After fixation, discs were rinsed three times in distilled water and stained with 2.5% aqueous uranyl acetate for 1 h at room temperature. Discs were then rinsed in distilled water and dehydrated through a series of 30, 50, 70, 90 and 100% (2×) ethanol and propylene oxide (2×), and then left for several hours to overnight at 4°C in 50% propylene oxide/50% epon resin. Discs were embedded in epon, which was allowed to polymerize at 43°C for 36 h and then sectioned along a line parallel with the dorsal–ventral boundary. When the elongated columnar epithelium of the wing pouch was reached, sectioning was monitored by drying 0.6-μm sections onto glass slides, staining them with toluidine and methylene blue, and observing them with phase contrast optics. When we observed that the plane of the sections had become parallel to the orientation of the cells, we saved the 0.6-μm section for reference and began to cut thin (0.07-μm) sections. These sections were stained with uranyl acetate and lead citrate and examined in an electron microscope (model 301; Phillips Scientific, Mahwah, NJ).

## *Results*

We wanted to disrupt Dcdc42 and Drac1 function in only a subset of the disc epithelial cells in order to allow the comparison of affected and unaffected cells in the same disc. For this reason, we chose to express mutant alleles of *Dcdc42* and *Drac1* under the indirect control of the *patched* promoter (see Materials and Methods). Fig. 1 shows the pattern of patched promoter activity in a third instar larval wing disc. It is active in a strip of cells just anterior to and abutting the anterior–posterior (AP) compartment boundary.

*Figure 1. Patched-gal4*-driven β-galactosidase expression in a late third instar wing disc. Flies harboring the *patched gal4* construct were crossed to flies carrying the gene for β-galactosidase under the control of the *gal4* UAS. Wing discs from late third instar larvae were fixed and incubated with X-gal to detect β-galactosidase activity. The dark stain marks the cells in which the *patched-gal4* construct is active. The broad stripe is located in the elongated columnar epithelial cells. The narrower stripe is out of the plane of focus and located on the other side of the disc at the interface between the squamous and columnar cells (see Fig. 2). Only the elongated columnar epithelial cells were examined for their response to Dcdc42 and Drac1 dominant-negative expression. A line is drawn through the wing pouch to indicate the approximate position of the sections shown in Fig. 2. Bar, 50 μm.

### *DCDC42 Is Required for the Elongation of Epithelial Cells*

Using this approach, we expressed one constitutively active and two dominant negative alleles of *Dcdc42*. Expression of the constitutive *Dcdc42V12* in the disc epithelium caused too much cell death to be informative, but expression of both the dominant-negative alleles, *Dcdc42S89* and *Dcdc42N17*, caused an ectopic furrow to form at the AP compartment boundary. We chose to characterize *Dcdc42S89*-expressing discs further because the *Dcdc42S89* phenotype was somewhat stronger.

To examine the changes in the cells that caused the furrow, we fixed and sectioned the affected discs along the line shown in Fig. 1 and stained them with methylene and toluidine blue. The wild-type disc comprises three different kinds of epithelial cells as shown in Fig. 2: the squamous cells of the peripodial membrane (*S*), columnar cells (*C*), and highly elongated columnar cells (*EC*). Since these cells are narrower than the nucleus over much of their length, the nuclei are packed at different levels, giving the epithelium a pseudostratified appearance (Fig 2 *A*). In the disc that expresses Dcdc42S89 at the AP boundary, cells that ought to be highly elongated are instead short, resulting in the deep furrow indicated by the arrow in Fig. 2 *B*).

To confirm that the furrow was formed by Dcdc42S89-expressing cells, we stained these discs with an antibody to cdc42. These experiments showed that Dcdc42S89 is overexpressed with respect to the endogenous level of Dcdc42, and that the region of the disc that expresses Dcdc42S89 forms the furrow (Fig. 3 *A*). Dcdc42S89 is localized predominantly to apical and basal regions of cells. In wild-type cells, the endogenous Dcdc42 also is more abundant in apical and basal regions, but has a more cytoplasmic and punctate distribution (Fig. 3 *B*). Under fixation conditions that include high levels of detergent (see Materials and Methods), Dcdc42 is depleted from the cytoplasm and per-

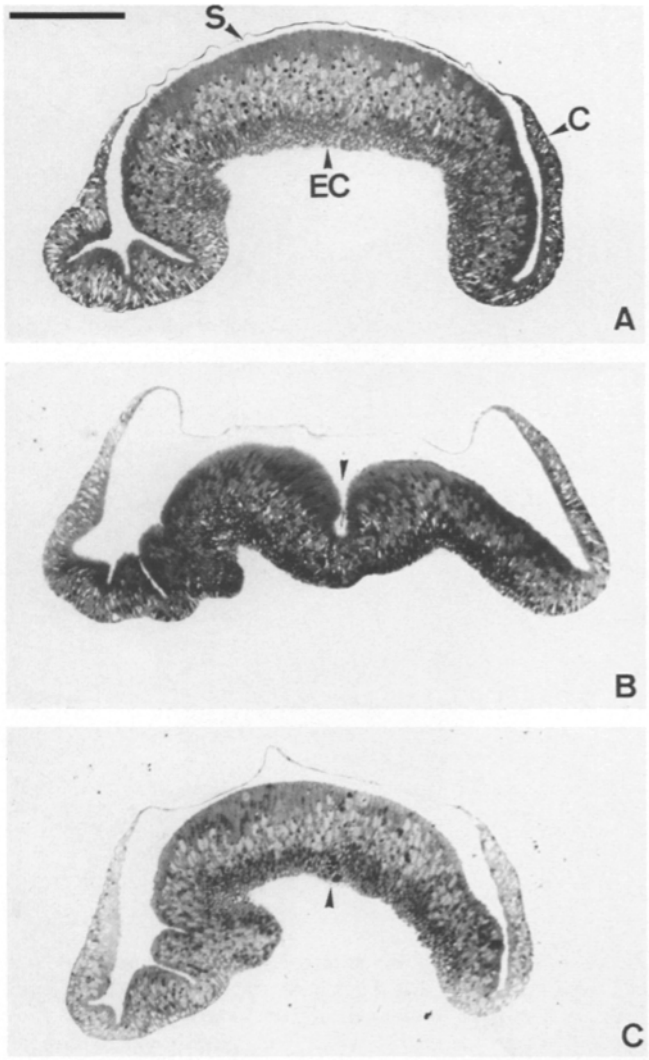



*Figure 2.* Cross-sections of (*A*) wild-type, (*B*) Dcdc42S89/ptc-gal4, and (*C*) Drac1N17/ptc-gal4 wing discs. The sections were stained with osmium tetroxide, toluidine blue, and methylene blue. The position of the sections corresponds to the line in Fig. 1. In *A*, *S* indicates the squamous cells, *C* the columnar cells, and *EC* the elongate columnar cells. The apical surface faces the lumen, and the basolateral surface faces out. In *B*, an arrow indicates the fold caused by *patched-gal4*–mediated Dcdc42S89 expression. In *C*, an arrow indicates dead cell s being extruded from the basal side of the epithelium. Bar, 50 μm.

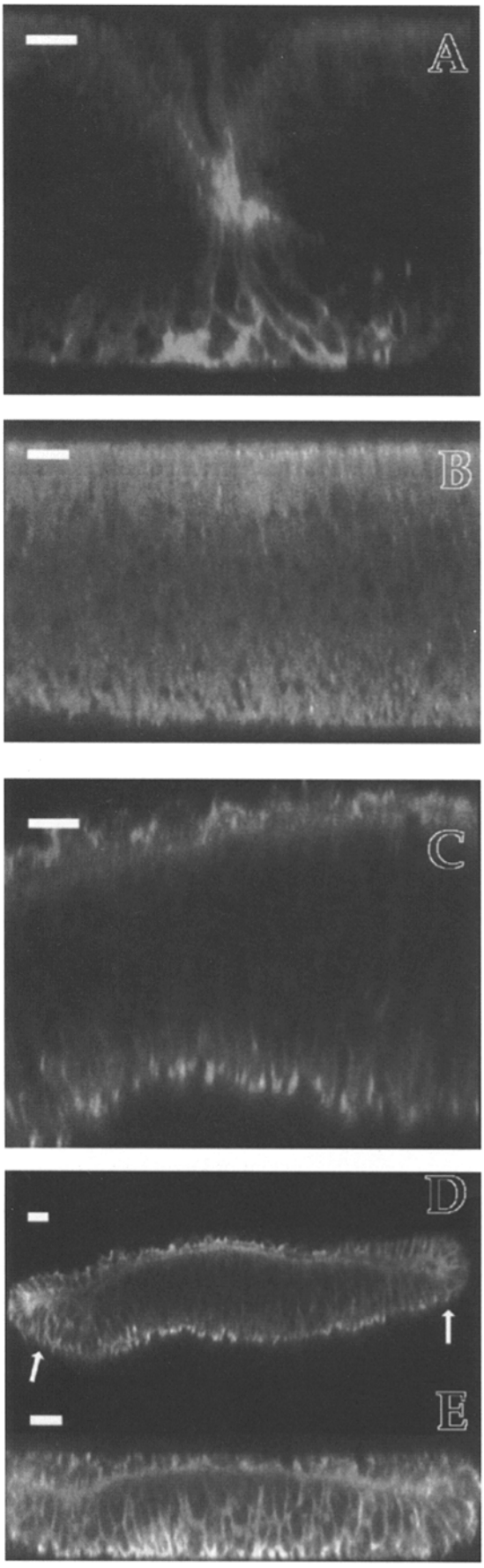
sists near membranes (Fig. 3, *C–E*), suggesting that a subpopulation of Dcdc42 interacts tightly with cellular components near membranes. In elongated cells, this subpopulation of Dcdc42 is restricted to the apical and basal membranes (Fig. 3, *C* and *D*). In contrast, cells in early third instar discs, which have not yet elongated (Fig. 3 *E*) and the columnar, but nonelongated, cells at the edges of late third instar discs (Fig. 3 *D*, *arrows*) display Dcdc42 over most of the lateral membrane as well. Thus, epithelial elongation correlates with depletion of Dcdc42 from lateral membranes and relative enrichment on apical and basal membranes.

To examine the disrupted cells in more detail, we processed affected discs for EM. In some experiments, we marked the Dcdc42S89-expressing cells with β-galactosidase and performed X-gal staining before processing to unambiguously identify mutant cells (data not shown). We could show that the interface between Dcdc42S89-expressing and -nonexpressing cells was often marked by an indentation on the basal surface of the disc (Fig. 4, *arrow*). To the right of the indentation one can see the basal regions of five undisrupted cells. These cells are quite slim basally and have basal filopodia (*arrowheads*). The disrupted cells to the left of the indentation, in addition to being abnormally short, are bloated basally relative to wild-type cells. Because filopodia are thin and easily missed by single sections even in wild-type cells, we have not attempted to quantify and compare their frequency in Dcdc42S89-expressing cells.

### *Elongating Cells Reorganize Their Basal Actin Cytoskeleton In a Process that Requires Dcdc42*

The apico-basal growth that produces the elongated columnar epithelium occurs during the third instar. In other cells that require cdc42 for polarized growth, the actin cytoskeleton plays an important role. To examine the role of the actin cytoskeleton in epithelial elongation, we stained early, middle, and late third instar wing discs with rhodamine phalloidin. Viewed in cross-section, discs at all three stages have abundant actin at the junctional region. In contrast, only the elongated cells accumulate significant amounts of basal actin (Fig. 5, *A–C*). A tangential optical section through the basal region of an early third instar disc shows that actin is located predominantly on the lateral membranes of the cells (Fig. 5 *D*). However, at later stages, actin disappears from the lateral membrane and is reorganized basally so that it has a more focused appearance (Fig. 5 *E*). If the basal reorganization of actin is important for elongation, then cells that do not elongate should not reorganize their basal actin. We therefore examined the columnar cells at the edges of the discs that remain short (labeled *C* in Fig. 2 *A*). Compared with the elongated cells (Fig. 5 *C*), the columnar cells do not accumulate significant amounts of basal actin (Fig. 5 *F*). The fact that the basal actin rearrangement occurs only in the elongating cells suggests that it plays a role in polarizing the shape of these cells.

The subcellular localization of Dcdc42 indicated that it might mediate elongation by acting at either the apical or basal surface (Fig. 3). To determine whether Dcdc42 was important for basal actin reorganization, we stained discs that expressed Dcdc42S89 at the compartment boundary with rhodamine-conjugated phalloidin. Before elongation has occurred to a significant extent, the Dcdc42S89-expressing cells are only slightly shorter than the adjacent cells, and the distribution of actin is not remarkably different, although it may be slightly more abundant laterally (Fig. 5 *G*). By the late third instar, however, normal cells have reorganized their basal actin, whereas the Dcdc42S89-expressing cells next to them have not (Fig. 5 *H*). Viewed in tangential section through the basal region, actin in Dcdc42S89 disrupted cells outlines the cell boundaries; in the normal cells to either side it appears more focused (Fig. 5 *I*). Dcdc42S89 does not disrupt localized actin accumulation in general; despite the extreme misorganization of the




basal actin cytoskeleton, accumulation of actin at adherens junctions is not affected (Fig. 5 *H*). Taken together, these findings suggest that Dcdc42 specifically promotes the reorganization of actin basally and that this reorganization is required for elongation.

### *Expression of Dominant-negative Dcdc42 Disrupts Basal Actin Plaques*

Fristrom and Fristrom (1975) observed via the electron microscope dense actin-containing plaques on the basal membrane of larval disc epithelial cells. Since we saw by staining with phalloidin that the basal actin cytoskeleton was disrupted by Dcdc42S89, we wondered whether these specific structures were affected. We therefore compared the basal region Dcdc42S89-expressing cells with those of the adjacent normal cells. We found that the basal membrane of normal cells contains abundant electron-dense plaques (Fig. 6 *A*, *arrows*) similar in morphology to those described by Fristrom and Fristrom (1975). Cells that express Dcdc42S89 have no such structures (Fig. 6 *B*). In the section shown in Fig. 3, we counted 12 electron-dense plaques along 12 μm of wild-type basal surface and only one small, abnormally condensed plaque in 12 μm of disrupted basal surface. The elimination of basal plaques by Dcdc42S89 suggests that Dcdc42 organizes the basal actin cytoskeleton by effecting the linkage of actin to the basal plasma membrane.

It is unclear whether these plaques represent classical focal adhesions, or whether they might play some other, nonadhesive role. Since integrins are often associated with basal focal adhesions, we asked whether the absence of the basal plaques correlated with a change in basal β-integrin distribution. The basal localization of β-integrin is not significantly altered in the disrupted cells (Fig. 7). This suggests that the basal plaques may not mediate adhesion to the extracellular matrix. Alternatively, an as yet unidentified integrin molecule may nucleate their formation.

### *Dcdc42 Controls Apical Cell Shape*

Since we detected Dcdc42 protein apically as well as basally, we thought that it might also play a role in organizing

*Figure 3.* Subcellular distribution of Dcdc42 and Dcdc42S89 proteins. (*A*) XZ confocal section of a disc expressing Dcdc42S89 at the compartment boundary stained with an antibody to murine cdc42. The Dcdc42S89 protein is enriched at the basal surface, at the apical surface, and in the cells that form the ectopic furrow. The sheet of cells seen extending into the Dcdc42S89-induced furrow is part of the squamous peripodial membrane. (*B*) XZ section through the wing pouch of a wild-type disc stained with anti-cdc42 antibody as in *A*. The gain is increased to reveal the weaker endogenous staining, which is punctate and cytoplasmic. (*C–F*) XZ sections through discs that have been detergent extracted concomitant with fixation and stained with an antibody to cdc42 (see Materials and Methods). (*C*) Wing pouch of late third instar disc. Detergent-resistant Dcdc42 is localized to apical and basal membranes. (*D*) Late third instar disc. Detergent-resistant Dcdc42 is present on the lateral membranes of nonelongated columnar cells (*arrows*), but is restricted to the apical and basal membranes in elongated cells. (*E*) Early third instar disc. Detergent-resistant Dcdc42 is present on all membranes. Apical is up, basal down. Bars, 5 μm.

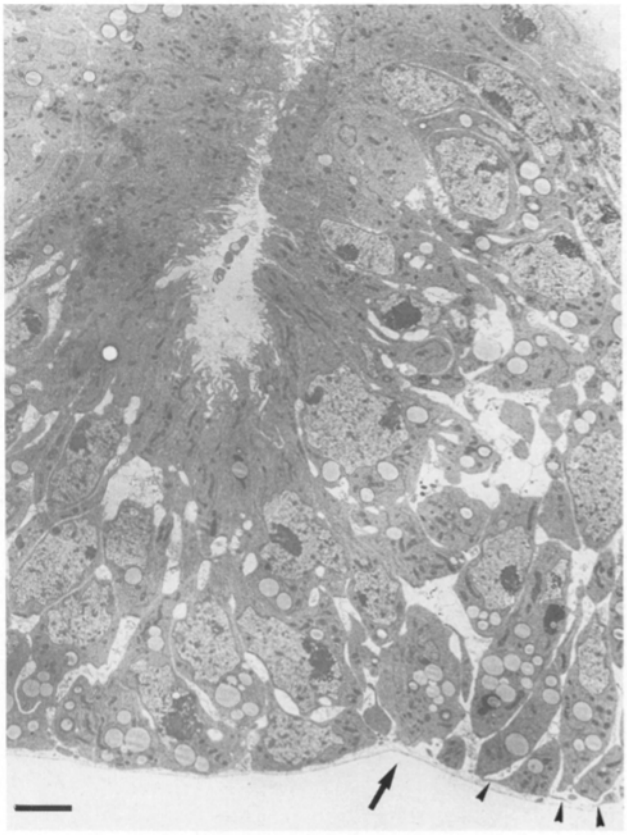


*Figure 4.* Electron micrograph depicting the region disrupted by Dcdc42S89 expression. This section is adjacent to the one shown in Fig. 2 *B*. The arrow indicates an indentation in the basal surface that correlates with the interface between Dcdc42S89-expressing and -nonexpressing cells. The cells to the left of the indentation have a broader basal surface than the cells to the right of the indentation. Arrowheads indicate basal filopodia. Apical is up, basal down. Bar, 1 μm.

the apical side of the disc epithelium. Although we were unable to detect obvious differences in the apical actin cytoskeleton in Dcdc42S89-expressing cells, the abundant apical junctional actin might have obscured any alterations. We therefore examined the apical regions of Dcdc42S89-expressing cells in the electron microscope to see if any defects were present at the ultrastructural level. In wild-type cells, the adherens junctions (Fig. 8, *arrows*) are very close to the apical lumen. They are rather convoluted and frequently oriented parallel to the apical membrane (Fig. 8 *A*). In contrast, the adherens junctions of the Dcdc42S89-expressing cells, although normal in morphology, are further from the apical side and are perpendicular to the apical surface (Fig. 8 *B*). It therefore seems likely that the apically located Dcdc42 helps to organize the apical shape of the cell.

### *Drac1N17 Does Not Affect Elongation or Basal Actin Reorganization*

To determine whether these alterations in cellular structure were specific to Dcdc42, we compared the effects produced by Dcdc42S89 with those caused by the dominant-negative Drac1 protein, Drac1N17. Histological examination of such discs showed that Drac1N17 expression does not produce a furrow, and cells appear to be of normal length (Fig. 2 *C*). This suggested that, unlike Dcdc42, Drac1 is not required for apico-basal elongation. Histological sections also showed that, despite their relatively normal appearance, Drac1N17-expressing discs often contain dead cells that appear to be extruded from the basal side of the epithelium (Fig. 2 *C*, *arrow*). Next, we examined the basal actin cytoskeleton in Drac1N17-expressing cells. Unlike cells disrupted by Dcdc42S89, these cells produce basal foci of actin, consistent with their ability to elongate normally (Fig. 9 *A*). Because these discs appear fairly normal by these criteria, and because of the cell death observed, we worried that Drac1N17 might kill cells as soon as it was expressed and that no Drac1N17-expressing cells might remain in the epithelium. Therefore, we asked whether Drac1N17-expressing cells were present in these discs. Wild-type discs stained with an antibody to Drac1 exhibit a punctate staining pattern that was uniformly abundant throughout the disc (Fig. 9 *B*). Examining discs bearing the UASDrac1N17 and GAL4 constructs revealed an induction of Drac1N17 expression along the compartment boundary. An optical cross-section through this region clearly demonstrates that Drac1N17-expressing cells are still present in these discs (Fig. 10 *A*). Drac1N17 is present throughout the cells where it is expressed. The hourglass shape of this region suggested to us that these cells are broader apically and basally than their neighbors, and somewhat more constricted in the middle. Overall, these cells seem to bulge slightly from both the apical and basal side, in stark contrast to Dcdc42S89-expressing cells. Since the defect caused by Drac1N17 expression was completely different from those produced by the Dcdc42 dominant negative, we concluded that the effects we observed in each case were specific. Nevertheless, since Drac1 is more closely related to Drac2 than to Dcdc42, we cannot exclude the possibility Drac1N17 interferes with the function of Drac2 as well.

### *Drac1 Is Necessary to Recruit Actin to Adherens Junctions*

When we directed our attention to other elements of the actin cytoskeleton, we realized that, unlike Dcdc42S89, Drac1N17 expression prevented the localization of actin to adherens junctions. The extent of Drac1N17 expression (Fig. 10 *A*) precisely correlates with the region where adherens junction actin is disrupted (Fig. 10 *B*). In some discs, lateral actin is also depleted; however, this effect is not consistently reproducible (data not shown). Disruption of adherens junction actin is also apparent in tangential optical sections through the apical junctional region (Fig. 10, *D* and *F*, *arrows*). Since the adherens junction actin in Dcdc42S89-expressing cells appeared normal (Fig. 5 *G–I*), we concluded that Drac1 was specifically required for its assembly.

### *Localized E-Cadherin and β-Catenin Cannot Recruit Junctional Actin in the Absence of Drac1 Activity*

The recruitment of actin to adherens junctions is initiated by the interaction of E-cadherin molecules on neighboring cells (Nagafuchi et al., 1987; Gumbiner et al., 1988). Actin

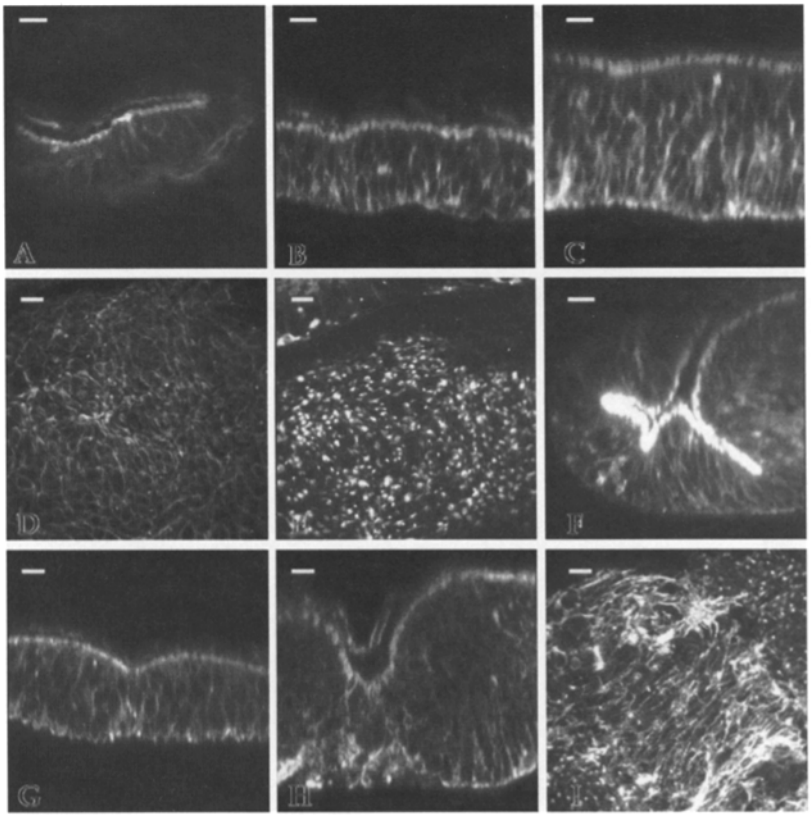


*Figure 5.* Actin cytoskeletal dynamics during elongation of wild-type and Dcdc42S89-disrupted cells. *A–I* Confocal micrographs of discs stained with rhodamine phalloidin to illuminate filamentous actin. *A–F* are wild-type discs. *G–I* are Dcdc42S89-expressing discs. (*A*) XZ section of an early third instar wing disc. Actin is abundant at the apical junctions. (*B*) XZ section of a mid-third instar wing pouch. Actin is abundant at apical junctions and begins to accumulate basally. (*C*) XZ section of a late third instar wing pouch. In addition to the actin at the adherens junctions, bright foci of basal actin are observed. (*D*) XY projection of the basal surface of an early-mid–third instar wing disc (three XY sections comprising 3 μm total depth were projected to obtain the entire basal surface in one picture). Actin predominantly outlines the lateral cell membrane. (*E*) XY projection (3 μm total depth) of the basal surface of a late third instar wing pouch. Actin is arranged entirely in a punctate pattern. (*F*) XZ section through the columnar, nonelongated cells (marked *C* in Fig. 2) at the edge of the late third instar disc shown in *C*. Very little basal actin is observed. (*G*) XZ section through the wing pouch of a mid-third instar disc expressing Dcdc42S89 at the compartment boundary in the center of the section. These cells are slightly shorter than their neighbors. (*H*) XZ section through the wing pouch of a late third instar disc expressing Dcdc42S89 at the compartment boundary. Focal basal actin is present in the normal cells to either side, but the cells at the boundary have predominantly lateral actin and are missing basal foci. (*I*) XY projection (3 μm total depth) of the basal surface of a late third instar disc expressing Dcdc42S89 at the compartment boundary. Note the absence of basal actin foci and the presence of lateral actin in the disrupted cells in the center of the disc. In *A–C* and *F–H*, apical is up, basal is down. Anterior is to the left and posterior to the right except for *A* and *D*, where dorsal is to the left and ventral is to the right. Bars, 5 μm.

does not bind directly to E-cadherin; its binding depends on two proteins, β- and α-catenin, which themselves bind to E-cadherin (Ozawa et al., 1990; Ozawa and Kemler, 1992; Aberle et al., 1994). We wanted to know whether Dracl was required for any of these interactions, or whether it played a novel role in the recruitment of actin. To determine whether Dracl was required for the localization of cadherin, we double-stained Dracl N17-expressing discs with an antibody to *Drosophila* E-cadherin (Fig. 10 *C*) and with phalloidin (Fig. 10 *D*). We found that, although cadherin is lost from the adherens junction in some cells, nevertheless many cells in which actin is no longer localized retain normal amounts of cadherin (compare Fig. 10 *C* and *D*). To determine whether Dracl was required for the localization of β-catenin, we double-stained discs with armadillo, the fly homolog of β-catenin, (Fig. 10 *E*), and phalloidin (Fig. 10 *F*). Again, it is clear that some cells that have lost actin still retain armadillo (compare Fig. 10, *E* and *F*). Indeed, armadillo accumulates to slightly higher levels in these cells than in their normal neighbors. We concluded from these data that the requirement for Dracl is either subsequent to or independent of the localization of cadherin and armadillo.

A further observation makes it likely that the requirement for Dracl is not independent of, but subsequent to, the localization of these proteins. We noticed that the adherens junction actin in the vicinity of the dorsal–ventral boundary was resistant to the depredations of Dracl N17 (Fig. 10, *D* and *F*). The dorsal–ventral boundary runs perpendicular to the AP boundary where Dracl N17 is expressed and intersects it in the middle of the disc (Fig. 10, *D* and *F*, *arrowheads*). The dorsal–ventral boundary cells express *wingless*, which causes the accumulation of armadillo protein (Riggleman et al., 1990; Peifer et al., 1991;

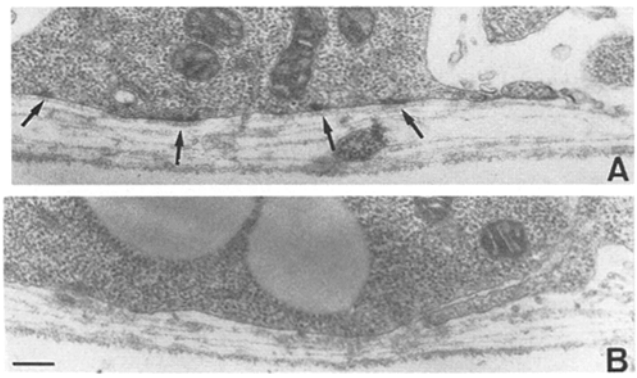


*Figure 6.* Basal electron-dense plaques are absent in Dcdc42S89-expressing cells. (*A*) Basal side of wild-type epithelium. (*B*) Basal side of a cell expressing Dcdc42S89. Arrows point to electron-dense plaques, which are absent in Dcdc42S89-expressing cells. Apical is up, basal down. Bar, 0.22 μm.

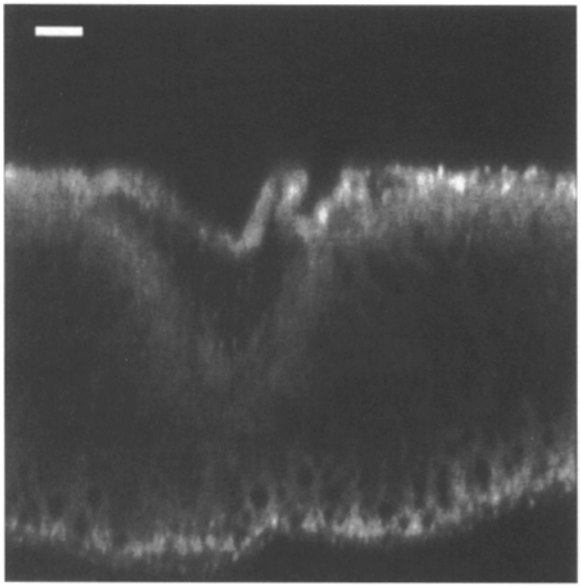


*Figure 7.* Integrin localization is unaffected by Dcdc42S89. Confocal XZ section of a disc expressing Dcdc42S89 at the compartment boundary stained with an antibody to β-integrin. The Dcdc42S89-expressing cells form the ectopic furrow (see Fig. 3). Anterior is left, posterior right. Bar, 5 μm.

van Leewen et al., 1994). The fact that increasing the level of armadillo can compensate for reducing Drac1 function suggests that these proteins participate in the same pathway and that Drac1 does not act independently of armadillo and cadherin.

### *Neither Dcdc42S89 Nor Drac1N17 Disrupts Polarized Protein Accumulation*

The actin cytoskeleton is thought to play a role in the polarized delivery of apical and basolateral proteins in epithelial cells, both directly (Achler et al., 1989; Fath and Burgess, 1993) and indirectly via its effect on the polarization of microtubules (Buendia et al., 1990). Our results thus far suggest that Dcdc42S89 and Drac1N17 disrupt different functional subsets of the actin cytoskeleton; Dcdc42S89 causes both apical and basal abnormalities, and Drac1N17 perturbs adherens junction actin. To determine which, if any, of these aspects of the actin cytoskeleton is involved in this process, we examined the effects of Dcdc42S89 and Drac1N17 on the distribution of apically and basolaterally located proteins.

Cadherin is normally present at high levels at the junctional region, which separates the apical and basolateral domains, and also localizes to spots on the basolateral

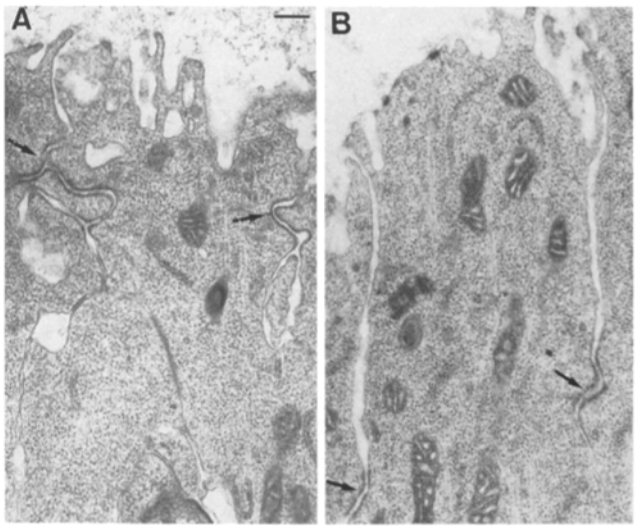



*Figure 8.* Dcdc42S89 expression alters the apical shape of epithelial cells. (*A*) Electron micrograph of the apical region of a wild-type cell. (*B*) Electron micrograph of the apical region of a Dcdc42-expressing cell. Arrows point to the adherens junctions. Apical is up, basal down. Bar, 0.22 μm.

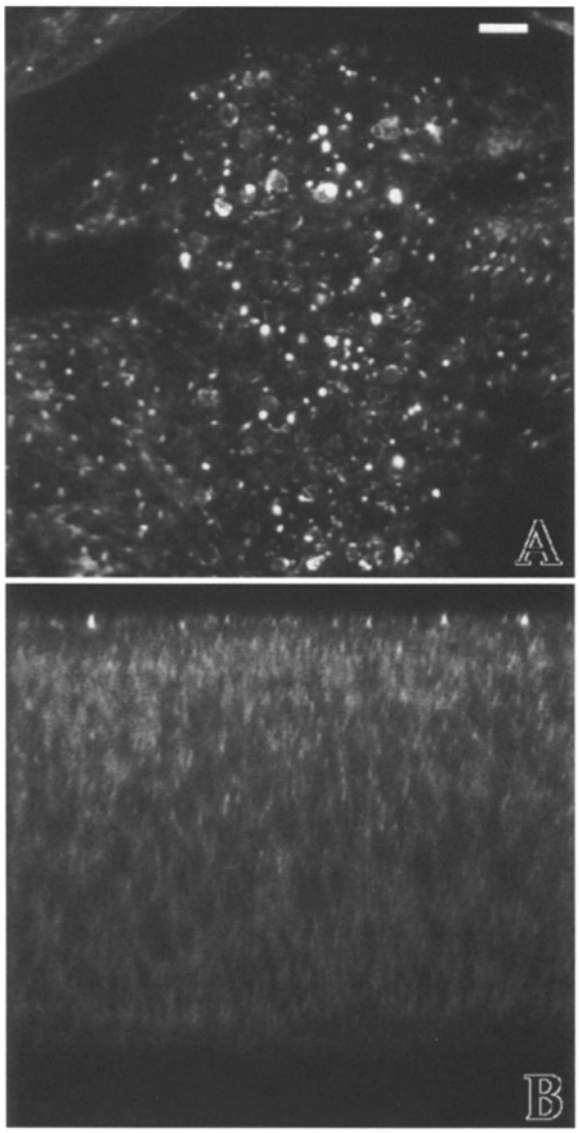



*Figure 9.* Basal actin organization is not altered by Drac1N17. (*A*) XY projection (3 μm total depth) of the basal side of a disc expressing Drac1N17 at the compartment boundary. The disc was stained with rhodamine phalloidin to detect filamentous actin. Basal actin in Drac1N17-expressing cells remains punctate. (*B*) XZ section of a wild-type disc stained with anti-rac1 antibody. Anterior is left, posterior right. Bar, 5 μm.

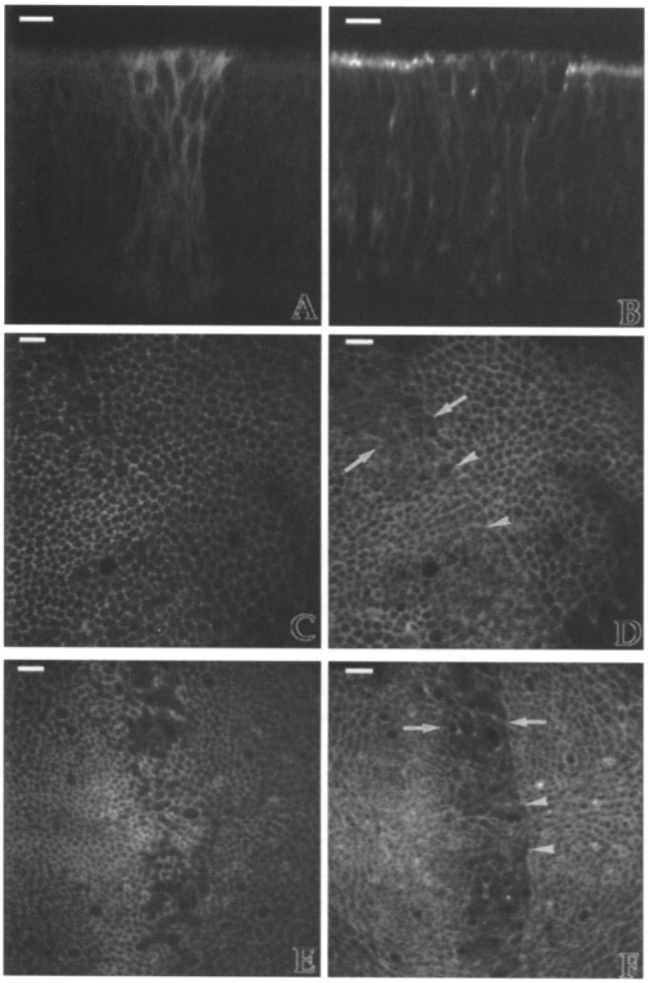


*Figure 10.* Adherens junction actin is disrupted in Dracl1N17-expressing cells. *A–F* are confocal micrographs of discs expressing Dracl1N17 at the AP compartment boundary. (*A* and *B*) XZ optical sections of a disc double-stained with an antibody to Dracl (*A*) and with rhodamine phalloidin (*B*). (*C* and *D*) XY projections (2 μm total depth) of the apical junctional region of a disc double-stained with an antibody to cadherin (*C*) and with phalloidin (*D*). (*E* and *F*) XY projections (3 μm total depth) of the apical junctional region of a disc double-stained with an antibody to armadillo (*E*) and with phalloidin (*F*). The arrows in *D* and *F* delimit the AP compartment boundary region where actin is disrupted; the arrowheads bracket the subset of cells at the intersection of the AP and dorsal/ventral compartment boundaries, where adherens junction actin is not disrupted. The fixation procedure used for the disc shown in *C* and *D* was different from the procedure used in *A*, *B*, *E*, and *F* (see Materials and Methods). These protocols optimize the staining of each antibody, but the preservation of actin differs. Preservation of the fuzzy-appearing actin in the mutant cells in *D* and preservation of the basal actin cytoskeleton (seen clearly in Fig. 5, but not in *B*) both depend on utilization of the fixation protocol described in Materials and Methods as being for "phalloidin alone." Anterior is left, posterior right. Bar, 5 μm.

membrane. Both Dcdc42S89- (Fig. 11 *A*) and Dracl1N17- (Fig. 11 *B*) expressing cells properly localize the cadherin protein to these regions. Furthermore, we are unable to detect any ectopic cadherin apical to its concentration in the junctional region. The yellow protein is normally localized apically, and there is no evidence of ectopic basolateral protein in cells disrupted by either Dcdc42S89 (Fig. 11 *C*) or Dracl1N17 (Fig. 11 *D*). Furthermore, examining the adult wing reveals that the cuticle, which comprises a host of apically secreted proteins, is normal in the wing generated by both Dcdc42S89- and Dracl1N17-expressing cells (Fig. 12, *C* and *E*). There is no evidence that cuticle has been secreted basally, where it would accumulate between the dorsal and ventral wing surfaces. These data suggest that neither Dcdc42 nor Dracl1N17 is required to specifically localize or maintain these proteins on the apical or basolateral surface. Furthermore, it suggests that neither Dracl-dependent adherens junction actin nor Dcdc42-dependent basal actin plays a continuing role in these processes.

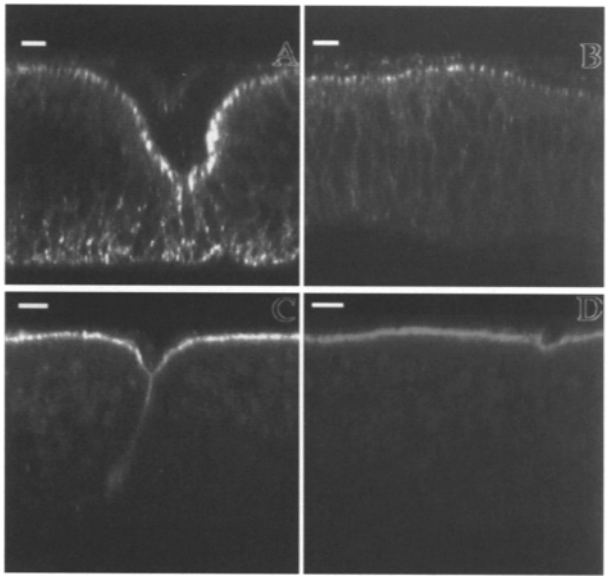


*Figure 11.* Neither Dcdc42S89 nor Dracl1N17 disrupts polarized accumulation of yellow or cadherin. Figure shows XZ confocal sections through the wing pouch of discs expressing either Dcdc42S89 (*A* and *C*) or Dracl1N17 (*B* and *D*) at the compartment boundary. *A* and *B* are stained with an antibody to E-cadherin. *C* and *D* are stained with an antibody to yellow. Apical is up, basal down, anterior left, posterior right. Bars, 5 μm.

### *Dcdc42 and Dracl Play Different Roles in Wing Morphogenesis*

Both cell shape and the actin cytoskeleton are highly dynamic during pupal wing morphogenesis when the final shape of the adult wing is attained. During morphogenesis, the elongated columnar cells of the wing pouch undergo profound changes in shape and are folded into an epithelial bilayer whose basal sides are apposed and adhere to each other via integrin-dependent focal contacts. Each cell in this bilayer extends a hair from its apical side. We wondered whether Dcdc42 or Dracl were involved in these processes as well. To address this, we examined the wings of adult flies and asked whether regions of the wing derived from Dcdc42S89- or Dracl1N17-expressing cells differed from other parts of the wing.

In 30% of flies that expressed Dcdc42S89 at the compartment boundary, the dorsal and ventral sides of the

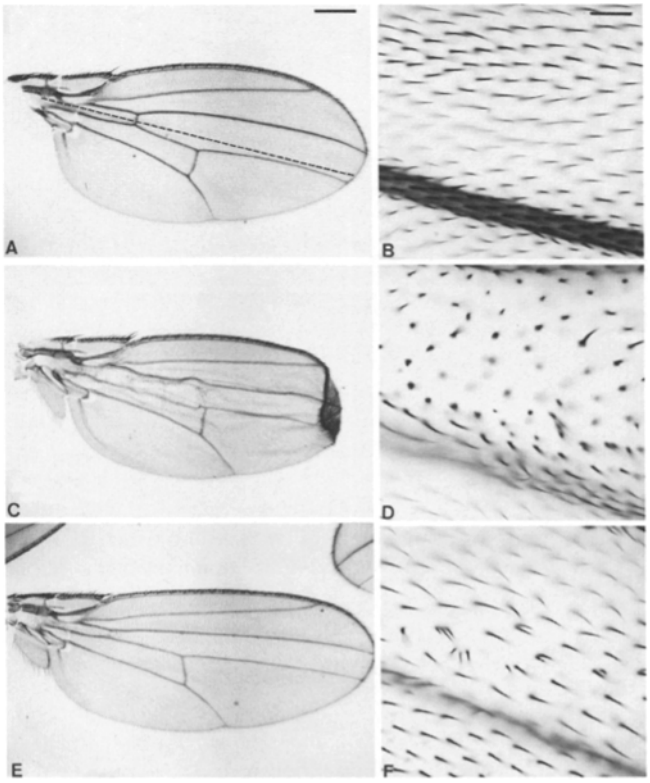



*Figure 12.* Effects of Dcdc42S89 and Drac1N17 on the adult wing. (*A*) Wing from a wild-type female. The dashed line indicates the boundary between anterior and posterior compartments (Garcia-Bellido et al., 1973). (*B*) Enlargement of the wild-type compartment boundary region. (*C*) Wing from a female expressing Dcdc42S89 at the compartment boundary. (*D*) Enlargement of the Dcdc42S89-affected region. The fuzzy spots are out-of-focus hairs on the opposite side of the wing. (*E*) Wing from a female expressing Drac1N17 at the compartment boundary. (*F*) Enlargement of the Drac1N17-affected region. In each case, anterior is up and distal is right. Bars: (*A*, *C*, and *E*) 250 μm; (*B*, *D*, and *E*) 31 μm.

wing come apart and form a blister in this region (Fig. 12 *C*). This suggests that the apposition or adhesion of the basal surfaces of the two epithelia is dependent on Dcdc42. The formation of blisters in the wing may reflect an epithelial elongation defect similar to that observed in the third instar, since apposition of the basal surfaces of the dorsal and ventral wing epithelia depends on elongation that occurs after pupariation (Fristrom et al., 1994). We also found that Dcdc42 is required for the formation of wing hairs in 100% of the flies examined. Comparing wings derived from wild-type (Fig. 12 *B*) and Dcdc42S89-expressing (Fig. 12 *D*) cells at high magnification reveals that the mutant hairs are either stunted or absent. The affected hairs are strictly limited to the compartment boundary region, but the penetrance is incomplete. Wings are most severely affected proximally, and cells near the wing margin rarely display abnormalities. Furthermore, the width of the band of cells affected (approximately four cells) is smaller than observed in the third instar disc, suggesting that penetrance also decreases anteriorly. This may reflect small differences in the level of dominant-negative expression in these cells, or a differential sensitivity to the dominant negative.

In contrast, Drac1N17 caused none of the defects produced by Dcdc42S89; there was no evidence of blistering (Fig. 12 *E*), and the wing hairs were of normal length (Fig. 12 *F*). Drac1N17 caused defects that were not produced by Dcdc42S89, however. Wings from these flies were of normal length but narrow. The reduction in area (of ~12%) was limited to the anterior compartment, which contains the cells that express Drac1N17. To determine whether the anterior compartment contained fewer cells than normal, or whether the cells had a narrower cross-section, we counted the wing hairs along a line from the compartment boundary to the anterior wing margin. We found that the number of wing hairs, and hence the number of cells, was abnormally low throughout the anterior compartment (data not shown). This supports the conclusion we made on the basis of the histology of these discs: Drac1N17 causes defects that result in higher rates of cell death.

When we examined wings at higher magnification, we noticed that Drac1N17 expression caused the duplication or triplication of wing hairs (Fig. 12 *F*). Again, the penetrance of this defect decreased near the wing margin. The wing margin corresponds to the dorsal–ventral boundary, which we have observed to be insensitive to the expression of dominant-negative Drac1 in the imaginal disc (Fig. 10, *D* and *F*). A similar insensitivity at later developmental stages may preclude the formation of multiple wing hairs near the wing margin. The formation of multiple wing hairs is a defining characteristic of the tissue polarity mutants. Tissue polarity genes are thought to polarize epithelial cells with respect to the body axis, a phenomenon termed planar polarization (for review see Adler, 1992). We therefore think that Drac1 contributes to this process.

## Discussion

In this study, we have perturbed different aspects of epithelial morphology by expressing dominant-negative alleles of Dcdc42 and Drac1 in imaginal discs. The specificity of the effects produced by Dcdc42S89 and Drac1N17 is remarkable. We did not detect any overlap in the function of these two molecules at any stage of development. Since the effects produced by dominant-negative Dcdc42 and Drac1 do not overlap, it is clear that neither nonspecifically disrupts the activity of the other. Furthermore, it is unlikely that Drho activity is altered because Dcdc42 and Drac1 share even less homology with Drho than they do with each other; Dcdc42 and Drac1 are 70% identical at the amino acid level (Luo et al., 1994), but share only 46% identity with DrhoA (Hariharan et al., 1995). Therefore, the phenotypes produced by Dcdc42S89 and Drac1N17 expression probably accurately reflect the roles of these proteins in cellular organization.

On the other hand, expression of dominant-negative forms of these proteins may not produce the equivalent of a null phenotype if the activities of the endogenous proteins are not completely eliminated. Although the basal actin cytoskeleton is most sensitive to reduction in the activity of Dcdc42, we cannot rule out the possibility that a low level of Drac1 activity (lower than that required for the assembly of adherens junction actin) is also needed.

Similarly, small amounts of Dcdc42, in addition to Drac1, might be required to assemble actin at adherens junctions. Nevertheless, the protein most likely to be regulatory for each process in vivo is the one to which it is most sensitive.

### *The Role of Drac1 In Adherens Junction Assembly*

Our results show that Drac1 activity is required for actin assembly at adherens junctions. Since assembly of actin is critical for cadherin-mediated adhesion, modulation of Drac1 activity in vivo might help regulate the ability of epithelial cells to adhere to each other. Drac1 does not seem to promote actin assembly by helping to localize either E-cadherin or β-catenin (armadillo) because Drac1N17 disrupts actin assembly in cells with properly localized cadherin and armadillo (Fig. 10, *C–F*). This suggests that Drac1 acts either downstream or independently of the localization of these proteins. Our data also suggest that failure to assemble actin in response to the localization of the cadherin–armadillo complex might result in the delocalization of cadherin or armadillo because we also observe cells that lack cadherin and armadillo as well as actin. As expected, we never observed cells with normal amounts of junctional actin but depleted cadherin or armadillo.

An intimate relation between armadillo and Drac1 is suggested by the observation that dorsal–ventral boundary cells, which contain higher levels of armadillo due to *wingless* expression, are resistant to the effects of expressing dominant negative Drac1 (Fig. 10, *C* and *E*). A simple explanation for this is that armadillo interacts directly with Drac1. The dominant-negative Drac1N17 protein binds nonproductively to armadillo, preventing a productive interaction with the endogenous, active Drac1. In cells with particularly high levels of armadillo protein, Drac1N17 might not be able to bind and sequester all the armadillo, leaving some protein free to interact with the endogenous, active Drac1. Similar suppression of the dominant inhibitory activity of Ha-ras N17 is obtained by expressing the exchange factor SDC25-C in yeast (Schweighoffer et al., 1993). Intriguingly, armadillo contains 12 repeats of a motif found in *smg*GDS (Peifer et al., 1994), an exchange factor for small GTPases including rac (Hiraoka et al., 1992). This raises the possibility that armadillo might act as an exchange factor for Drac1.

Recently, Harden et al. (1995) have reported the effects of heat shock–induced expression of Drac1N17 on embryonic development. They found a failure in dorsal closure that correlated with altered cell shape and decreased filamentous actin at the leading edge of the dorsally migrating epithelial cells, but no defects in adherens junction actin were apparent. Differences between the expression systems probably account for this discrepancy; when Drac1N17 is expressed under the indirect control of the *patched* promoter, the dominant-negative protein is present throughout ongoing cell division, when new adherens junctions must be assembled. Taken together, our results and those of Harden et al. suggest that Drac1 probably plays multiple roles in regulating actin accumulation.

### *The Survival of Normal Epithelial Cells May Require Intact Adherens Junctions*

A wide range of experiments with tissue culture cells has established that the integrity of adherens junction components is critical to adhesion, and that tumorigenesis correlates with their inactivation (Navarro et al., 1991; Vleminckx et al., 1991; Matsuyoshi et al., 1992; Behrens et al., 1993). On this basis we might have expected that disc cells expressing Drac1N17 would fail to adhere to the rest of the epithelium and lose their single-layered structure. We saw no evidence of this; instead, we observed increased levels of cell death. Cell death was seen both histologically (Fig. 2 *C*) and deduced from the reduction in the size of the wing (Fig. 12 *E*). One possible explanation for increased mortality is that Drac1N17 disrupts another vital cellular function, such as cytokinesis. Alternatively, disassembly of the adherens junction itself may be toxic. Interestingly, epithelial cells mutant for *dco*, in which no adherens junctions are detectable in the electron microscope, also exhibit high levels of cell death and retain a monolayered structure (Jursnich et al., 1990). It is possible that death may be a common response of normal, nonimmortalized epithelial cells to compromised adherens junction function. This response would share strategic features with anoikis, a term coined to describe epithelial apoptosis that occurs as a result of detachment from the extracellular matrix (Frisch and Francis, 1994). Both responses would ensure that continued growth occurs only when cells are constrained within an epithelium. It is clear that a cell's ability to evade anoikis is one requirement for full transformation. Our results suggest that this may also be true in the case of cell–cell contact; for tumorigenesis to progress, cells must not only abrogate contact with their neighbors; they must circumvent the apoptotic response that results.

### *Drac1 Helps to Generate Planar Polarity*

Another difference between the developing disc and cells in tissue culture is that disc cells develop obvious planar polarity, that is, polarity with respect to the body axis, as well as apico-basal polarity. In the wing this is reflected in the regular array of distally pointing hairs, one of which is elaborated by each cell on the wing blade. By disrupting Drac1 function in vivo, we have been able to observe that generation of planar polarity in the disc epithelium apparently requires Drac1.

The realization that epithelial cells are polarized with respect to the body axis came from the study of the *Drosophila* tissue polarity mutants. This class of mutants perturbs the normally well-ordered arrays of cuticular hairs and bristles (Adler, 1992). The tissue polarity genes affect many epithelial tissues, but efforts to understand their mechanism of action have focused on the wing. In the wing, planar polarity within each epithelial cell is reflected in the choice of the site at which hair outgrowth initiates (Wong and Adler, 1993). The hair begins as an actin-filled apical projection that is restricted to the distal-most vertex of the cell (i.e., the point at which it contacts its two most distal neighbors). Some tissue polarity mutants make wing hairs that do not point distally. In these cells, the actin-filled outgrowth can occur anywhere on the apical surface of the cell and is not limited to a site of cell–cell contact. Other tissue polarity mutants, like Drac1N17, cause a single cell to form multiple wing hairs (Fig. 12 *F*). These cells fail to limit the initiation of actin polymerization to the dis-

tal vertex; instead, actin accumulates at multiple regions of cell–cell contact. This phenotype is very different from that observed by Dcdc42S89 expression, which causes either the absence of wing hairs or stunted hairs (Fig. 12 *D*). One simple explanation for the requirement for Dracl in the establishment of planar polarity is that specification of the distal vertex is signaled by some modification of the adherens junction.

### *Dcdc42 Controls Cell Shape Changes in Epithelial Cells*

The control of epithelial cell shape changes is of critical importance to morphogenesis and differentiation. Despite the importance of these events, very little is known about how epithelial cells change their shape. This work establishes that Dcdc42 mediates epithelial elongation and suggests that elongation depends on Dcdc42-mediated changes in the basal actin cytoskeleton. Although Dcdc42 is probably also required to shape the apical region (Fig. 8), we do not observe any apical cytoskeletal changes that correlate with elongation during the third instar. Adherens junction actin cannot be required for the maintenance of an elongated shape, because Dracl N17-expressing cells that lack it are of normal height. Furthermore, adherens junction actin accumulates normally in Dcdc42S89-expressing cells (Fig. 5 *H*). In contrast, we find that the basal actin cytoskeleton is organized differently in elongating versus nonelongating cells, and that this organization is dependent on Dcdc42. Cells in which the basal actin cytoskeleton is disrupted by Dcdc42S89 expression are missing electron-dense plaques on their basal plasma membrane, suggesting that Dcdc42 may act by promoting the linkage of microfilaments to the membrane in this region.

Interestingly, other types of metazoan cells also use cdc42 to generate different kinds of actin-dependent polarized shape changes. Neurons require cdc42 for neurite extension (Luo et al., 1994), and fibroblasts respond to cdc42 activation by extending filopodia (Nobes and Hall, 1995). These processes occur in different cellular contexts, nevertheless the common requirement for cdc42 activity suggests that they all share a basic mechanism. Since the generation of polarized cell shape changes in fibroblasts and neurons has been intensively studied in tissue culture, information gleaned from these studies might shed light on the mechanism by which epithelial cells change their shape.

### *Are Filopodia Involved in Dcdc42-mediated Epithelial Cell Shape Changes?*

Both fibroblast migration and neurite extension depend on the formation of filopodia. In fibroblasts, these dynamic, actin-filled projections seek out new contacts with the substrate, and are subsequently connected by the actin-dependent formation of lamellipodia. Contraction results in the breakage of old attachments and net forward movement of the cell (Small, 1994; Cramer et al., 1994). Filopodia also form in growth cones when neurites are elongating. Here, they perform a similar function and search out new connections between the growing neurite and its substrate (Bentley and O'Connor, 1994; Doherty and Walsh, 1994). In fibroblasts, activating cdc42 promotes filopodium formation (Kozma et al., 1995; Nobes and Hall, 1995); in neurons, perturbation of cdc42 activity prevents neurite outgrowth (Luo et al., 1994). In light of these facts, a straightforward explanation for the failure of neurite outgrowth is that cdc42 is required for filopodium formation in neurons as well. This suggests the intriguing possibility that Dcdc42-dependent epithelial elongation and wing hair formation are similar processes and also involve filopodia. Basal filopodia have been described in a variety of epithelia including the sea urchin archenteron (Morrill and Santos, 1985; Keller and Hardin, 1987), the ectoderm of the insect *Calpodes* (Locke and Huie, 1981), and cultured vertebrate cells (Vasiliev, 1987; Reinsch and Karsenti, 1994). We also observe filopodia in normal disc epithelial cells (Fig. 4 and Fig. 6 *A*).

Basal filopodia in epithelial cells contact both the extracellular matrix and neighboring cells, suggesting that they may be able to generate focal contacts with either depending on the exigencies of the task at hand. When epithelial cells elongate, they must increase the percentage of their surface area in contact with neighboring cells. Under these conditions, filopodia may favor contacts with other cells over contacts with the extracellular matrix. Subsequent retraction of filopodia into the body of the cell might result in an increase in height (Locke and Huie, 1981). Clearly, such a mechanism requires a specific and intricate arrangement of the actin cytoskeleton, and this may be reflected in the basal reorganization of actin that occurs when cells elongate. This mechanism also demands coordination of the processes occurring in neighboring cells to bring about an increase in the height of the apposed lateral surface membranes.

### *Dcdc42 And Drac1 In Epithelial Polarity*

The defects caused by dominant-negative Dracl and Dcdc42 expression do not affect the maintenance of epithelial polarity in general, because apical and basolateral proteins remain correctly localized. We were particularly surprised by the observation that Dracl N17 did not affect polarity, because adherens junction actin is thought to help polarize the organization of epithelial microtubules (Buendia et al., 1990). It may be that the increased rate of cell death caused by Dracl N17 expression interferes with our ability to observe depolarization; if cells in which the adherens junction is fatally compromised undergo apoptosis, they may die before apical and basolateral proteins are observably delocalized.

These experiments address the function of Dcdc42 and Dracl in the imaginal epithelium, but the generation of epithelial polarity occurs much earlier, during embryogenesis (Knust, 1994). Although Dcdc42 and Dracl N17 appear not to be required for the maintenance of polarity, they may yet play a part in its generation. Manipulating the activity of Dcdc42 and Dracl at the time that polarity is being established may reveal a different set of functions for these versatile proteins and provide insights into these fascinating changes in cell structure.

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